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Conservation and Utilization of Genetic Resources in Canada

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Abstract

Canada manages a distributed repository system for plant, animal and microbe genetic resources, integrated to increase efficiency through commonality of functions. This public-good service-oriented research provides high quality germplasm and well documented information to national and international clients dealing with crop and livestock production, improvement and bio-based product development. The research is specific to the conservation and utilization of genetic resources. Genetic resources and associated information are believed to play a fundamental role on an ongoing basis to most fields of biological science because, they provide insurance of supplying basic materials for agriculture and agri-food against future adverse conditions; preserve germplasm of useful traits such as resistance to new pests, adaptability to climate change, other environmental conditions or compounds for bio-based products; inputs into inspection and regulatory matters; and provide stability to farming systems at the local, national and international levels by smoothing yield variability through maintenance of a wide range of diversity. All these ultimately impact Canada's ability to trade internationally. Canada's research effort in the conservation and utilization of genetic resources is worthy as generates positive policy, environmental and social impacts. As biodiversity has been identified as an important contributor to society's mental and physical well-being, being able to conserve and describe the genetic diversity and variation of plants, animals and microbes plays a major role in support of this well-being.

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Introduction

Canada through Agriculture and Agri-Food Canada, has managed and conserved genetic resources for more than 37 years. Plant Gene Resources of Canada (PGRC) initially located in Ottawa, Ontario and re-located in 1998 to the Saskatoon Research Centre, Saskatoon, SK was established in 1970 with the mandate to protect, preserve, and enhance the genetic diversity of plant species of economic importance and their wild relatives. The Clonal gene bank at the Greenhouse and Processing Crops Research Centre, Harrow, Ontario followed a few years later as did the Potato Repository at the Potato Research Centre, Fredericton, New Brunswick. Management responsibility for two microbial collections, the Canadian Collection of Fungal Cultures at the Eastern Cereal and Oilseed Research Centre, Ottawa and the Plant Virus collection at the Pacific Agri-Food Research Centre in Summerland, British Columbia were added. The most recent addition to the national program in May 2006 was a domestic Animal Genetic Resources program co-located in the University of Saskatchewan (Western College of Veterinary Medicine and the Animal and Poultry Science Department), Saskatoon. In this distributed repository system stretching across Canada, about 50 professional and technical staff members have expertise in crop breeding, horticulture, entomology, plant pathology, population genetics, reproductive physiology, cryobiology and bioinformatics. All basic functions of gene banks are conducted including acquisition, distribution, maintenance, regeneration, characterization, evaluation and documentation. Efficiencies of operation and management have been obtained through integration of plant, animal, microbe and plant virus collections.

During summer/fall, 2006 the Federal Minister of Agriculture conducted a series of consultations across Canada to determine the science priorities for the government and Agriculture and Agri-Food Canada. Consultations were obtained from a wide audience including farm producers, marketers, academics, provincial extension personnel, public and private agri-industry representatives. Seven priority areas were identified:

1. Enhanced human health and wellness through food and nutrition and innovative products;
2. Enhancing the quality of food and the safety of the food system;
3. Enhancing security and protection of the food supply;
4. Enhancing economic benefits for all stakeholders;
5. Enhancing environmental performance of the agricultural system;
6. Understanding and conserving Canadian bioresources;

Science deliverables:

* availability of information on the classification/attributes of bioresources
* availability of science based tools and knowledge to predict the behavior of biological resources in response to environmental change

7. Developing new opportunities for agriculture from bioresources

From these consultations a peer review research proposal process was developed to encompass all research being conducted by AAFC. The Canadian Genetic Resources Program obtained strong positive comments on its proposal and was full funding for 4 years. Recently the Animal Genetic Resources program has received new and additional funding through a Risk Mitigation initiative. The Program clearly fits under national Priority #6 with the following long-term and short-term objectives.

Long-term objectives are to protect and conserve the genetic diversity of Canadian bioresources, contribute to the security, protection and safety of the food system, enhance the environmental performance of the Canadian agricultural system, contribute to the development of new opportunities for agriculture, thereby enhancing food and feed quality, Canadian health and wellness, and economic benefits for the industry, and support bioresource-related regulatory requirements. Short-term objectives are to: a)develop new techniques to conserve and regenerate plant, animal and microbial germplasm to maintain genetic integrity and minimize genetic erosion; b)create new phenotypic and genotypic information including identifying new sources of disease resistance, abiotic stress resistance, nutritional quality and bioactive compounds, through characterization and evaluation of bioresource attributes; c)assess genetic diversity changes in domesticated plant and animal germplasm; d)improve the structure of the GRIN-CA database for delivery of bioinformation; and e)contribute to access and benefit sharing regimens (acquire, donate, maintain, regenerate germplasm) consistent with Canada's commitments to international treaties, e.g. Convention on Biological Diversity (CBD) and the FAO International Treaty on Genetic Resources for Food and Agriculture (ITGRFA).

The Program objectives have high relevance to AAFC priorities: They are aligned with, and create the basis for, enhancing our understanding of Canadian bioresources, and protecting and conserving their genetic diversity (Priority 6). Deliverables include the development of information and technologies associated with acquisition, conservation, regeneration, characterization, evaluation and documentation of plant, farm animal and

microbial bioresources. This research is essential to the conservation of Canadian bioresources, to ensuring a supply of diverse germplasm to face adverse conditions, to the development of adaptation strategies to climate change, and to support regulatory requirements. The objectives contribute to: a) developing new opportunities for agriculture from bioresources, and enhancing human health and wellness through food, nutrition and innovative products (Priorities 7, 1), through development of information on the composition, structure and possible applications of bioresources that can contribute to the bioeconomy; b) enhancing economic benefits for industry and environmental performance of the Canadian agricultural system (Priorities 4, 5), through identification of useful germplasm which will contribute to cultivar improvement, the reduction of production risk factors, and the reduction of pesticide use, thus improving management practices and reducing soil and water contamination from pathogens and pesticides; and c) enhancing the quality and supply of food and the security, protection and safety of the food system (Priorities 2, 3), through contributions to the development of tools, techniques and strategies for the detection and mitigation of microbial threats to the safety, security and protection of Canadian food production and distribution systems. This meets Canada's legally binding commitments to international treaties and conventions such as the Convention on Biological Diversity (CBD) and the International Treaty on Genetic Resources for Food and Agriculture (ITPGRFA).

Current holdings

Plant Gene Resources of Canada (PGRC) maintains, and conducts research on an *ex situ* collection of about 110,800 accessions of plant germplasm at: a) Saskatoon (1050 plant species: 107,000 accessions); b) Harrow (fruit trees and small fruits: 3,500 accessions); and c) Fredericton (potatoes: 139 clones); the Canadian Collection of Fungal Cultures maintains 14,000 strains, the Canadian Plant Virus Collection maintains 550 isolates of 230 different viruses and the Animal Genetic Resource Program already has over 250,000 doses of semen and 85 embryos from 16 cattle breeds, over 1100 semen doses from elk and bison, over 1400 blastodermal cells from 42 poultry lines and about 500 semen samples from chicken and turkey breeds.

Development of new techniques to conserve and regenerate plant, animal and microbial germplasm to maintain genetic integrity and minimize genetic erosion

Outdoor germination experiment on 16 native Canadian plant species included germination in a replicated experiment using 4 soil types. A study was continued on the application of germination promoters (including GA_3, KNO_3, and ethylene) for the germination of native plant seeds; initiated the development of a protocol using the vital dyes fluorescein diacetate and sytox green for the determination of seed viability.

Continued evaluations of fabric tents for isolation increase of crucifer germplasm. Tents constructed with durable material require less maintenance than commercially available tents and provide some frost protection for later maturing germplasm.

New protocols for production of *Sclerotinia sclerotiorum* ascospore under controlled conditions were developed along with a new protocol for inoculation of *Brassica napus* with ascospores on petals of *Arabidopsis taliana*.

Research was initiated on in vitro produced pig embryos. Agreements were developed to obtain a constant supply of ovaries from a local abattoir.

Collaborations were initiated with scientists at the University of Saskatchewan studying bison reproduction and the development of a new extender to improve sperm cryopreservation in cattle. With the bison group two grant proposals were successfully submitted related to the application of reproductive technologies for bison and on *in vitro* fertilization to evaluate semen frozen with different extenders.

A new mechanism of programmed cell death in bull sperm was developed. Sperm proteins harvested from fresh and frozen semen of different bulls, and egg yolk containing extender were subjected to SDS electrophoresis and 2-D electrophoresis to quantify heat shock protein-70 and caspase-3, proteins involved in somatic cell death. Both proteins are present in fresh and frozen sperm, but their patterns varied. The expression of caspase-3, and executioner protein, increased significantly after freezing and processes. This phenomenon was further investigated and found that egg yolk contributes a significant amount of caspase-3 to sperm cells during freezing process. This study will help to understand the defence mechanism in mammalian that will ultimately lead to improve the sperm longevity in female tract by adding inhibitor proteins. It will ensure the presence of

spermatozoa for the fertilization of oocytes. This study will help the AI industry to produced high quality semen with maximum production potential.

Vitrification of bovine oocytes: The cryopreservation of female gametes (oocytes) is not as successful as in male gamete (sperm) due to their biochemical and structural differences, yet protocols are being developed for the vitrification of bovine oocytes. The effect of packaging system and equilibration time on vitrified bovine oocytes was investigated. Batch freezing (>3 bovine oocytes in straw or cryotop) could be done instead of single oocytes. The data revealed as high as 40% (48% of control) vitrified oocytes can be successfully matured *in vitro*. This study will help to conserve the female genetic material and produce offspring especially from endangered species.

Create new phenotypic and genotypic information including identifying new sources of disease resistance, abiotic stress resistance, nutritional quality, and bioactive compounds, through characterization and evaluation of bioresource attributes

AFLP analysis of 900 plains rough fescue plants collected from 30 populations across the Canadian Prairies was completed. Data processing and analysis are in progress. Final project report will be generated and delivered to Parks Canada (collaborator). From this research, we developed a marker-based approach to interpolating individual genetic information from a sample of plants to the whole population and empirically demonstrated the effectiveness of this approach for mapping genetic distinctiveness within 6 fescue populations across the Canadian Prairies. This approach is expected to play an important role in management and conservation of plant populations at risk.

Sequencing of 76 Canadian hard red spring wheat cultivars at Lr21 locus was completed. DNA sequences of about 4000 bp representing the Canadian wheat cultivars were completely aligned. Different analyses of diversity are in progress.

AFLP analysis of 40 Chinese flax cultivars and exotic germplasm was performed in collaboration with a Chinese visiting scientist (Dr. M. Li). Analysis of AFLP data is still in progress. Significant research findings may be written up for scientific publication.

1440 progeny rows of six *Camelina sativa* populations were grown, harvested individually to evaluate variation in seed oil content (NIR analysis) and were scored for severity of Aster Yellows disease.

Protocols were developed to screen barley accessions for seedling resistance to net blotch and spot blotch diseases. Cultures of net blotch and spot blotch strains were obtained from AAFC Winnipeg, and the National Fungal Collection. These cultures were regenerated and initial applications of inoculum made to barley plants in the greenhouse.

Characterization of chickpea germplasm for resistance to the fungal pathogen *Ascochyta rabiei* (ascochyta blight): 12 chickpea lines were screened for resistance to 48 *A. rabiei* isolates from Canada, Syria, India and Spain; five lines were highly resistant (immune) to all isolates; two lines showed a quantitative level of resistance to all isolates (minor R genes); three lines showed specific host-pathogen interactions (major R genes); while two lines were susceptible to all isolates; this data provides evidence of both quantitative and qualitative resistance. Genetic linkage maps of SSR and SNP markers were developed for 3 RIL populations derived from different resistant lines, and highly significant quantitative trait loci (QTLs) contributing to ascochyta resistance were identified.

A characterization nursery for *Dalea* (native Canadian plant) was established with the aid of collaborators. The nursery consisted of 8 lines, 5 of which originated from the native plant collection of PGRC. The 5 accessions were: NCP 368 – *Dalea* sp.; NCP 556 *D. candidum*; NCP 558 *D. candidum*; NCP 585 *D. purpurea*; NCP 591 *D. purpurea*. The remaining 3 accessions were the threatened species *D. villosa* (DV1; DV2; DV5). *Psoralea*: The collection contains 12 Psoralea accessions. Three accessions were used in an outdoor germination bed trial established in 2005. Germination counts made during the summer found a mean germination rate of NCP 50 (*P. esculenta*) at 1.25% in 2006 and 14.8% in 2007. NCP 95 (*P. lancelota*) had a germination rate of 13.1% in 2006 and 0% in 2007 and NCP 90 (*P. argophylla*) had a rate of 5.9 % in both 2006 & 2007.

Breeding strategies based on random bred and pedigreed populations in combination with repeated mating of sires allows for no rise in inbreeding until all sires mate with each dam or dam group. This strategy safeguards against loss of genetic variation and mean performance. The approach has potential merit in maintaining genetic authenticity of endangered breed populations.

Complementing North American sheep breeds that lack the inherent potential for increased fecundity and milk yield with the Finnsheep and Romanov as well as the newly developed Arcott breeds in Canada demonstrated outstanding lamb and ewe productivity as well as milk yield in long term trials at the University of Minnesota.

To taxonomically determine viral bioresources a search of the NCBI GenBank Database for *Carlavirus* sequences was performed to develop a database of sequence data as a foundation from which broad-spectrum RT-PCR primers could be designed. The GenBank data were sorted and organized in a DNASTAR Lasergene 7 database. Complete genomes were separated from the multitude of partial genomes which were further sorted by the region of the genome they span. Complete genomes are used for primer design and partial genomes for primer validation and phylogenetic analysis.

GenBank sequence files with complete viral genomes were selected for primer design. These consisted of 19 individual sequences representing 11 species in the *Carlavirus* genus. They were organized by genes and/or regions. Coding regions were organized by the corresponding translated protein. Sets of all homologous sequences were compiled in the FASTA format and aligned using the ClustalX and/or ClustalW algorithms. A primer set was used extensively to discover and collect information on new strains of the *Carlavirus*, Blueberry scorch virus (BlScV), which has been causing economic losses in the blueberry industry of BC. The virus is unusual as significant genetic diversity exists among the isolates examined from BC. The Carla–ORF2-3-F/Boeanchor primer set worked well in detecting all strains of the virus and of giving easily detectable RT-PCR results directly from blueberry plant extracts.

Assess genetic diversity changes in domesticated plant and animal germplasm

Analysis of genetic diversity in Sable Island horses was completed. Several North American horse breeds associations have shown interest in developing collaborative agreements to further characterize the genetic diversity within and between several horse breeds registered in Canada and the USA.

Initiated a genetic diversity study in Canadian pig breeds and commercial lines, the Canadian swine industry is concerned about the possible erosion of genetic diversity in their breeding stocks. Good relationships exist with breeders of minor pig breeds (Tamworth and Large Black) in collecting pig bristles for molecular characterization.

Research proposals to 11 major dairy and beef cattle breeds registered in Canada were submitted. The objectives are to monitor temporal changes in genetic diversity from genealogical records and multiloci DNA-based markers. Complete databases of pedigrees and DNA profiles from the Canadian Angus Association, the Canadian Limousin Association

and the Canadian Simmental Association have been received. Holstein Association of Canada has approved the research. The Canadian Hereford Association, the Canadian Charolais Association and the Canadian Maine-Anjou Association are waiting final approvals from their respective Board of Directors. Seven American beef and dairy breeds came forward and requested proposals for consideration by their Board of Directors. Maxxam Analytics Inc, a major DNA-based service provider to 20 different beef and dairy cattle associations and horse breed associations in North America, has fully endorsed the research proposals and has committed to freely share their multiloci DNA-based genotypes with CAGR.

Improving the structure of the GRIN-CA database for delivery of bioinformation

Plant genetic resource database

Considerable programming effort brought the GRIN-CA database into compliance with Government of Canada Common Look and Feel guidelines. Many front-end tables were updated for accuracy, completeness and link ability. Unfortunately CLF only makes use of about 2/3's of the width a full computer screen which results in an unattractive appearance for the GRIN-CA database. An initial database replication program was designed and is currently being tested for accuracy.

The GRIN-CA database was operational 365/24/7. No significant down time or computer malfunctions occurred. Since GRIN-CA became operational, over 20.5M hits have occurred and at a rate of over 14,000 requests / day made from over 120 countries.

Digital images of potato, large fruits and small grain accessions were added to GRIN-CA Passport data for clonal crops continued to be incorporated into GRIN-CA along with updates and corrections for many accessions. Characterization data was added for oat.

Animal genetic resource database

Development of an animal genetic resource database was initiated in cooperation with Brazil and the USDA (National Centre for Germplasm Preservation, Ft Collins). Discussions centred on content for 29 tables which ranged from inventory, taxonomy, donors, characterization and pedigree information, quality of donation, programming language, and hardware and software usage. Detailed programming is nearing completion, will be shared and integrated among the 3 countries. Windows XP and Unix Sun Solaris are being used

and the following software packages are being learned and utilized for database development: Oracle, MYSQL, Ruby, Ruby on Rails, JavaScript, Perl, PHP, TOAD, DBvisulizer, Oracle Form Builder, Oracle Database 10g Express Edition, Netbeans and Aptana Studio.

The current animal database includes three components: collection, repository and sample distribution. Sample collection contains sample donation and shipment information; the repository stores animal samples according to animal storage rules and procedures, and distribution reassigns current samples based on different requests (for research, evaluation, or working). Hierarchical structure was used in some tables to indicate the relationship between the upper-level and lower-level structure. The animal database was constructed on Oracle a RDM System and is in a Unix Sun Solaris server.

Contribute to access and benefit sharing regimens (acquire, donate, maintain, regenerate germplasm) consistent with Canadian commitments to international treaties, e.g. Convention on Biological Diversity (CBD) and the FAO International Treaty on Genetic Resources for Food and Agriculture (ITGRFA)

Germplasm from different sources was acquired. 361 samples from a joint Ukrainian-Canadian collection mission were completed. Wheat germplasm received from Bulgaria, Ukraine and the USA represented 164 accessions. Oat lines with a wide range of fat content (60 accessions) were obtained. Other acquisitions included: *B. napus*: 80 accessions **–** Nordic Gene Bank; 80 accessions **–** Czech Republic; 98 bean (*Phaseolus vulgaris*) **–** Ethiopia. The Clonal Repository acquired: four *Malus*, three *Prunus* and 21 *Fragaria* accessions. Two modern Canadian potato cultivars and five heirloom cultivars were added to the potato collection. 20 accessions were grown for agronomic evaluation, tuber glycoalkaloid analysis and plant, flower and tuber photographs in support of plant descriptions. One older cultivar and one heirloom cultivar were found to have glycoalkaloid levels above the threshold considered acceptable for human consumption.

Canada has signed and ratified the International Treaty on Plant Genetic Resources for Food and Agriculture. PGRC began using the Standard Material Transfer Agreement of the ITPGRFA in June 2008 which covers all plant germplasm held in Canadian collections. PGRC distributed germplasm within Canada and to 22 countries in 108 shipments. These shipments included 4850 accessions. About 49% of accessions were sent to Canadian clients, 33% sent to the USA and the remaining 18% to other countries. The Clonal

Repository distributed 607 accessions in 49 shipments involving 17,882 propagules. During the year 49 requests were received for potato accessions involving 552 clones. All but three requests came from Canada.

More than 65% of the PGRC collection is currently backup in safe secure duplication with the USDA at Ft. Collins, Co. In addition, PGRC sent about 6000 accessions, representing about 90 species to the Svalbard Global Crop Seed Vault in Norway as part of an international effort to safeguard global genetic resources. The Clonal Repository collected 2000 seeds from 2 *Prunus* accessions and 2000 seed from 400 *Malus* accessions for safe, duplicate back up at Saskatoon.

About 855 germination tests covering 20 genera were conducted. Over 31,000 germination tests conducted during 1998~2007 were entered into the GRIN-CA database.

For regeneration field plots, isolation sites, foliage tunnels (2) to increase heat units and prolong growth, greenhouse and growth cabinets are used. The following were increased in 2007: dry beans (39 accessions), faba beans (3), soybean (8), lettuce (4), Sorghum millet (7), *Pennisetum* millet (6), *Panicum* millet (1), buckwheat (1), egg plant (1), *Quinoa* (1) and sesame (8), mungbean (141 accessions), tobacco (2), wheat (141), sunflower (250), Jerusalem artichoke (358), cultivated flax (108), wild oat (436), cultivated oat (505), rye (87), barley (3500), wild barley (120), triticale (11), 45 accessions of *Poa alpina, P. arctica,* and *P. glauca* from the high Arctic and tomato (37), perennial oat, melons, coriander, cucumber, cantaloupe, pumpkin, winter squash, wild cucumber, *Echinacea,* buckwheat, bottle gourd, perennial flax, lupine and pea germplasm. About 60 alfalfa, 25 bird's-foot trefoil and 310 crucifer accessions were regenerated within pollinator excluded cages using leafcutter bees for pollination.

Detailed information about the PGRC lentil, chickpea, faba bean, grass pea, potato, barley, oat and wheat collections were compiled and provided to the Global Crop Diversity Trust, Rome for development of global plans for conservation of these crops.

Wild plant species surveys and seed collection in BC, Alberta, Saskatchewan and Manitoba included: a) 63 field days, 12,000 km of road travel, plant species surveys and seed collection from 71 locations; b) caging of native orchid species to prevent seed predation in collaboration with Manitoba Native Orchid Conservation; c) revision of the data collection form for seed collection; d) revision of a fact sheet on seed collection for

collaborators; and e) collection of 327 accessions of native plants (including seed from 41 uncommon or rare species) with data suitable for future use with GIS technology.

A Collaborative Agreement with Semex Alliance (world leader and provider of dairy cattle genetics and germplasm) was agreed. The CR calls for the transfer of about 200,000 straws of semen from close to 3000 dairy bulls to the CAGR germplasm collections. This represents a donation of approximately $5 M. It is anticipated that over the next 10 years, an additional 280,000 straws of semen will be transferred to CAGR representing another donation of $7 M.

Isolates of various *Carlavirus* species were obtained from other collections: (German) Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). These isolates were revived from their storage state, as freeze-dried infected tissue, by preparing an inoculum and using it to infect herbaceous indicator plants. These were monitored for symptoms and were passaged serially to increase the virus titre as needed. Species specific RT-PCR primers sets were obtained for each virus either from literature searches or by designing sets based on available sequence data.

The Clonal Repository hosted the WERA20 2007 meeting. WERA deals with virus and virus like diseases of fruit trees, small fruit and grapevines nationally and internationally.

A senior undergraduate course in plant genetic conservation and utilization was offered by staff in the Plant Science Department, University of Saskatchewan. Animal genetic resource staff offered several lectures in animal science and conservation within the Animal/Poultry Science Department and the Western Veterinary College, University of Saskatchewan.

Other productivity: Peer reviewed scientific publications: 20; Presentations: 26; Posters: 12; Technical reports, abstracts, book reviews, book chapters, books: 39; Tours: 24; Exhibitions and demonstrations: six (especially potato); Training: three Post-docs, 2 Ph.D. students, five MSc students, numerous short visitors.